REMARKS

The Examiner is respectfully requested to enter this Reply After Final in that it raises no new issues. Applicants have cancelled claims, and the cancellation of claims and claim amendments made herein were made to conform with the rejections under 35 U.S.C. § 112 and objections due to nonelected subject matter (as incorporated in the claims before the present amendment).

Alternatively, the Examiner is respectfully requested to enter this Reply After Final in that it places the application in better form for Appeal.

Applicants note that the previous rejection under the judicially created doctrine of obviousness-type double patenting, and the rejections under 35 U.S.C. §§ 101, 112, second paragraph, 102(b) and 103(a), are withdrawn (as listed on page 2 of the Office Action).

Claims 8 and 9 were previously cancelled. Claims 3, 5, 17, 19 and 25-40 were previously withdrawn from consideration. Claims 3, 5, 17, 19 and 25-42 are cancelled herein. Thus, claims 1, 2, 4, 6, 7, 10-16, 18 and 20-24 are pending in the present application.

Claims 1, 6, 7, 15 and 21 have been amended. No new matter has been added by way of these amendments, because each amendment is supported by the present specification. For example, the amendments to claims 1, 6 and 15 remove non-elected subject matter (i.e., SEQ. ID. NOS.:2 and 3) (see Restriction Requirement of April 25, 2001) and address issues of proper antecedent basis. The amendments to claims 7 and 21 were made

to change the dependency of the presently pending claims and are merely editorial in nature. Thus, no new matter has been added.

Based upon the above considerations, entry of the present amendment is respectfully requested.

In view of the following remarks, Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

Election/Restrictions

Applicants have amended and cancelled the appropriate claims to comply with the Restriction Requirement (dated April 25, 2001).

Claim Objections

The Examiner has objected to some of the pending claims for reasons of record (Office Action dated August 14, 2001). Applicants respectfully traverse.

Applicants have amended the appropriate claims, including claims 1 and 7, to comply with the Restriction Requirement (dated April 25, 2001), whereby SEQ. ID. NOS: 2 and 3 are no longer recited in the claims.

Further, claims 3, 5, 17, 19 and 25-42 have been cancelled. Thus, Applicants respectfully request that the Examiner withdraw this objection.

Issues Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 1, 2, 6, 7, 10-14, 15-16, 18, 20-24 and 41-42 under 35 U.S.C. § 112, second paragraph because of the use of the claim language "the protein" and "said protein". Applicants respectfully traverse.

The appropriate claims (*i.e.*, claims 1 and 15) have been amended to no longer recite "the protein", but instead now recite "a protein". Further, claims 41-42 have been cancelled, rendering the rejection of these claims moot. Thus, Applicants respectfully request the Examiner to reconsider and withdraw this rejection.

Issues Under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 1, 2, 4, 6, 7, 10-14, 15-16, 18 and 20-24 under 35 U.S.C. § 112, first paragraph, for the reasons cited on pages 4-7 of the Office Action. Applicants respectfully traverse these rejections, because the specification has adequate written description and provides sufficient enablement for one having ordinary skill in the art to make and use the present invention.

The Present Specification Adequately Describes the Present Invention

Applicants have shown in the specification that the claimed DNA fragment does encode a protein that is responsible for conferring resistance to PPO herbicides, whereby substitution at Val13 of the amino

acid sequence of PPO of *C. rheinhardtii*, or of the corresponding amino acid sequence, provides resistance to such herbicides.

Applicants have described the wild-type sequences of PPO genes and enzymes from *Chlamydomonas reinhardtii*, *Zea mays* and *Arabidopsis thaliana* (SEQ. ID. NOS:10, 13 and 11, respectively)[1]. wrong - this only encodes the 'Xho-PmaCL2.6' fragment, derived from the HindIII fragment

Furthermore, Applicants have described that a mutation at the residue corresponding to Val13 in the *Chlamydomonas* sequence, and to Val365 of the *Arabidopsis* sequence, is a mutation that confers resistance to PPO herbicides (*i.e.*, Example 10 confirms the amino acid sequences, and Example 11 identifies the mutations responsible for the herbicidal resistance).

Still further, Applicants have described that a domain encompassing the Val13 residue in *Chlamydomonas* PPO is also present in the region of the Val365 residue in *Arabidopsis* (see Example 11; in particular, Val13 in wild type *C. reinhardtii* PPO corresponds to Val365 in the *Arabidopsis* PROTOX gene as discussed at page 56, lines 31-33). This domain is described as SEQ. ID. NO.:1 (amino acids, SEQ. ID. NO.:4 provides an example of an encoding nucleotide sequence) in the present application. This domain represents a common feature of the proteins encoded by the DNA as instantly claimed. PPO herbicide resistance was then confirmed, as exemplified in Examples 12-13.

but val 13 is the position of the sequence encoded by SEQ ID not the entire protein.

[1] Only the nucleotide sequence is provided for *Chlamydomonas*, but the amino acid sequence can be easily obtained by translation.

The full length sequences of the PPO proteins set forth in the Sequence Listing, together with the description of the common domain in each that represents the site at which a mutation has been shown to confer PPO-herbicide resistance constitute adequate written description of a "protein comprising the amino acid sequence of SEQ ID NO: 1" that has PPO activity and a mutation conferring PPO-herbicide resistance.

Further, in considering the structure-function correlation of the DNA fragment recited in claim 15, Applicants respectfully request the Examiner to consider the homologous recombination of the DNA fragment.

The homologous recombination obviates the necessity to correlate the structure of the DNA fragment itself with protoporphyrinogen oxidase activity. This is because introducing the DNA fragment by homologous recombination provides the plant or plant cell with a DNA encoding a full protein having the protoporphyrinogen oxidase activity, even if the introduced DNA fragment itself encodes merely a part of the protein.

When introduced via homologous recombination, the DNA fragment is exchanged with the endogenous DNA present at the homologous loci in the plant cell or plant. A DNA fragment encoding even a part of the protein can, for example, be exchanged with the endogenous DNA at the homologous loci in the endogenous PPO gene. The resulting PPO gene encodes a protein having PPO activity and the function of conferring a resistance to protoporphyrinogen oxidase-inhibiting herbicides. The specification describes at page 50, lines 26-37, that the particle gun method can be

utilized to introduce via homologous recombination the Xho/PmaC2.6 fragment, in order to confer the herbicidal resistance.

Thus, the claimed isolated DNA fragment does encode a part of a protein, wherein the protein has protoporphyrinogen oxidase activity in plants, and has the ability to confer resistance to protoporphyrinogen oxidase-inhibiting herbicides in plant or algal cells when expressed therein (*i.e.*, as recited in claim 15).

Considering the biological deposit, submitted sequence listings, and experimental confirmation of structure and function, Applicants have demonstrated to one having ordinary skill in the art that they had possession of the claimed invention at the time of filing the present application.

One Skilled in the Art Can Readily Make and Use the Present Invention

In addition, Applicants also submit that because the DNA fragment, amino acid sequence, and any protein comprising the claimed DNA fragment have been sufficiently described, one having ordinary skill in the art would also be able to make and use the present invention without any experimentation that could be considered as undue.

Further, "[t]he initial burden of establishing a *prima facie* basis to deny patentability to a claimed invention on any ground is always upon the examiner." *See Ex parte* Parks, 30 USPQ2d 1234, 1236 (citing In *re Oetiker*, 24 USPQ2d 1443 (Fed. Cir. 1992)). Establishment of a *prima facie* case of nonenablement requires the Examiner to provide "acceptable

evidence of nonenablement". *See Utter v. Hiraga*, 6 USPQ2d 1709, 1714 (Fed. Cir. 1988).

Applicants respectfully submit that the burden of proving enablement has not shifted to the Applicants because the Examiner has failed to provide a reasoned statement and acceptable evidence as to why the present specification provides inadequate enablement to one having ordinary skill in the pertinent art. Applicants specification describes how to make and use the instantly claimed DNA fragment (see Applicants' remarks from Amendment of January 14, 2002) without undue experimentation.

Further, a proper analysis for enablement would consider all of the *Wands* factors, which include: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *See In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Considering these factors, a proper analysis and weighing of all factors tips in the Applicants' favor.

In considering the *Wands* factors, the present specification does show how to make and use the present invention. Those remarks pertain to what DNA and amino acid fragments can be used, and how they are employed to confer PPO herbicidal resistance in plants. For the convenience of the Examiner, those remarks are repeated below:

2. *Enablement*

The Examiner has also rejected claims 1, 2, 4, 6-16 and 20-24 under 35 U.S.C. § 112, first paragraph, as allegedly failing to enable one of ordinary skill in the art to make and use the invention. Specifically, the Examiner states that the specification does not teach a person skilled in the art to make and use the invention that is commensurate in scope with the claims, nor does the specification "reasonably provide enablement for any DNA fragment of encoding a part of the protein having PPO activity in plants" (see Office Action, page 12). Reconsideration and withdrawal of this rejection are respectfully requested.

First, Applicants note they have amended the appropriate claims.

Second, Applicants respectfully submit that the specification provides sufficient enablement to make and use the multitude of DNA fragments. The specification clearly teaches in Example 7 (see pages 47-51) that the DNA fragments can be produced by conducting a restriction digestion with DNA fragments that encodes the amino acid sequence having protoporphyrinogen oxidase activity. The rejection above sets forth no considerations of why the restriction digest is insufficient to fulfill the requirement for "making" the DNA fragments. The above rejection likewise sets forth no consideration as to why the specification is insufficient to fulfill the requirements for "using" the DNA fragment. Still, Example 5 of the specification clearly teaches a particle gun method so that one of ordinary skill in the art can employ the particle gun methods to confer herbicidal resistance to *Chlamydomonas*. Clearly, this is at least one use of the DNA fragments, which one of ordinary skill in the art can perform without undue experimentation.

Also, the specification provides sufficient enablement to make and use the claimed methods. The specification clearly enables one of ordinary skill in the art in introducing DNA fragments into plant cells. As described, the particle gun method would sufficiently enable one of ordinary skill in the art to introduce the DNA fragments into plants and plant cells for expression. Thus, the specification clearly enables the present invention.

Therefore, for the above reasons, the Applicants respectfully request the Examiner to reconsider and withdraw this rejection.

With regard to *Wands* factors (4) and (8), the nature of the invention involves insertion of a novel strand of DNA or amino acid sequence that will confer PPO herbicide resistance in a plant. More specifically, the presently pending claims are directed to cloned DNA conferring herbicide resistance and a method of conferring resistance to protoporphyrinogen oxidase-inhibiting herbicides upon plants or plant cells, comprising introducing the DNA fragment (or a plasmid containing the DNA fragment) into plants or plant cells or algal cells, wherein said DNA fragment is expressed. The DNA also has the certain characteristics. Those characteristics are (a) hybridization to a reference nucleic acid under recited conditions, (b) the DNA fragment encodes a part of a protein providing protoporphyrinogen oxidase activity in plants, and (c) the Val13 residue of SEQ ID NO:1 (or the corresponding residue from other species) is substituted by another amino acid so that the plant or algal cells develop resistance to protoporphyrinogen oxidase-inhibiting herbicides. Thus, the involved technology includes genetic engineering of plants and cloning and screening of nucleotide sequences for a desired activity.

For *Wands* factor (6), the skilled artisan will possess an advanced degree and typically will have post-doctoral experience. The skilled artisan is trained to conduct research and possesses technical skills

in building DNA libraries and cloning and screening of DNA for a desired activity.

For *Wands* factor (1), the quantity of experimentation necessary is exemplified by the present specification. The specification depicts Examples of constructing the cDNA library (Example 1), including that of *Chlamydomonas reinhardtii* (Example 5), and screening for the desired PPO-inhibiting herbicide resistance gene (Example 6). The nucleotide sequences of the DNA fragments can be determined by the method of Maxam and Gilbert of Sanger or improved versions of the method (see page 22, lines 23-30). In other words, the quantity of experimentation is expected in the art and is not undue.

For *Wands* factors (2) and (3), the specification provides ample direction or guidance by working examples and other disclosure in the present specification. For example, the PPO-resistant strain RS-3 is publicly available (see specification at page 15, lines 8-12 for this information), and sequence listings are given. With this starting material, one having ordinary skill in the art would be further guided by the present specification to prepare a cDNA library from the plant material of interest, and then identify clones which are able to supply PPO activity to a mutant host organism deficient in this activity (for example, see page 16, lines 13-18). Example 12 at page 57 shows how to create herbicide-resistant PPO genes by site directed mutagenesis of cloned DNA obtained in Example 2.

An assay for herbicide resistance conferred by this PPO activity of a protein encoded by a cloned DNA is described in, *e.g.*, Example 8 at page 52. Testing of transgenic plants for herbicide resistance is demonstrated in Example 17 at page 62.

Standard transformation methods can then be employed to give plants or plant cells the desired herbicide resistance (see Example 17, starting at page 62, showing results between wild-type versus transformed plants). These methods are further described in the specification with many additional working examples (for example, see pages 24 and 59-62). Furthermore, such methods and labor are merely routine in the art.

Once the plants or cells have such herbicide resistance, a determination of the nucleotide and/or amino acid sequence of the mutant obtained is merely routine in the art as well. Typically, such determinations involved comparisons to known wild-type proteins or DNA sequences (*i.e.*, see Example 10 on page 54). Thus, one having ordinary skill in the art would have sufficient direction and guidance to begin with the disclosed starting material, give a plant the desired herbicidal resistance, and determine the nucleotide sequence and corresponding amino acid sequence that confer such resistance. All of these procedures are also considered as not undue to one having ordinary skill in this art.

With regard to *Wands* factors (5), the state of the art is such that screening of cloned DNA for a desired activity is expected.

Furthermore, as noted above, either such screening methods are known in the art or are described above. Such screening will be able to sort working embodiments from those that are inoperable.

As to the unpredictability factor (7), Applicants submit that the Examiner is applying this factor from an improper viewpoint. The Examiner takes a position that, because it is not possible to predict *a priori* whether any particular nucleotide sequence encodes a protein having an activity according to the invention, the present invention is not enabled. However, the real issue is whether or not the skilled artisan can, beginning from what is explicitly described in the specification, predictably obtain another working embodiment, and another embodiment, etc., to fill the scope of the claims. Applicants submit that, by the library screening described in the specification, it is entirely predictable that the skilled artisan can practice the present invention in the full scope of the claims.

The Examiner should consider that, in *Wands*, the applicant's experimental results were such that only 2.8% of the hybridomas that were screened proved to be operable within the claims. Nevertheless, the Federal Circuit found that undue experimentation was not required to practice the claimed invention. The Court reasoned that screening was a step in practice of the invention that a skilled artisan expected to perform, and the specification described how such screening should be performed to sort operable from inoperable embodiments. *See In re Wands*, 8 U.S.P.Q.2d at 1405-06.

As applied to the present application, screening and testing are expected to have to be performed, and the present specification describes what experiments must be performed to effect the screening. Thus, such experimentation is not undue.

In summary, the experimentation required to practice the invention is only that which is either routine in the art, or is sufficiently described, including a showing by working examples of how to go from described starting materials to conferring herbicidal resistance in plants. It is fully predictable that, by practicing the invention as described in the specification and applying what was known in the art at the time the invention was made, the practitioner of the art having the normal level of skill can obtain working embodiments of the invention throughout the scope of the claims. Therefore, the present specification should be considered enabling of the claimed invention and the instant rejection should be withdrawn.

Based on the above remarks, Applicants respectfully request the Examiner to reconsider, and to withdraw all rejections and allow the currently pending claims.

A full and complete response has been made to the Office Action. The Examiner is respectfully requested to pass the application to issue.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Eugene T. Perez (Reg. No. 48,501) at the telephone number of the

undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Attached hereto is a marked-up version of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Version with Markings to Show Changes Made

(Rev. 02/20/02)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 3, 5, 17, 19, 25-42 have been cancelled.

The claims have been amended as follows:

1. (Twice Amended) A method of conferring resistance to protoporphyrinogen oxidase-inhibiting herbicides upon plants or plant cells, comprising introducing a DNA fragment or a plasmid containing the DNA fragment into plants or plant cells or algal cells, wherein said DNA fragment is expressed and has the following characteristics:

(1) said DNA fragment encodes a part of [the] a protein, wherein said protein has protoporphyrinogen oxidase activity in plants;

(2) said DNA fragment has a sequence that can be detected and isolated by DNA-DNA or DNA-RNA hybridization to a nucleic acid sequence that is complementary to a nucleotide sequence encoding an amino acid sequence [selected from the group consisting] of SEQ ID NO:1, [SEQ ID NO:2 and SEQ ID NO:3,] wherein said DNA-DNA or DNA-RNA hybridization occurs under 2X PIPES buffer, 50% deionized formamide, 0.5% (w/v) SDS, 500µg/ml denatured sonicated salmon sperm DNA at 42°C overnight; and said DNA fragment remains hybridized after washing in 2X SSC, 1% (w/v) SDS;

(3) said DNA fragment encodes the part of the protein in which an amino acid corresponding to Val13 of SEQ ID NO:1, [or SEQ ID NO:2 or SEQ ID NO:3] is substituted by another amino acid; and

(4) said DNA fragment has an ability to confer resistance to protoporphyrinogen oxidase-inhibiting herbicides in plant or algal cells when expressed therein.

6. (Twice Amended) The method according to claim 1, wherein [the DNA fragment encodes a protein or a part of the protein, wherein] said protein has protoporphyrinogen oxidase activity in *Chlamydomonas*[, and the DNA fragment encodes the protein or the part of the protein in which an amino acid corresponding to Val13 of SEQ ID NO:1 is substituted by another amino acid].

7. (Amended) The method according to any one of claims [1 to 6,] 1, 2, 4 or 6, wherein Val13 or the corresponding amino acid is replaced by methionine.

15. (Twice Amended) An isolated DNA fragment which has the following characteristics:

(1) said DNA fragment encodes a part of [the] a protein, wherein said protein has protoporphyrinogen oxidase activity in plants;

(2) said DNA fragment has a sequence that can be detected and isolated by DNA-DNA or DNA-RNA hybridization to a nucleic acid sequence that is complementary to a nucleotide sequence encoding an amino acid sequence [selected from the group consisting] of SEQ ID NO:1, [SEQ ID NO:2 and SEQ ID NO:3,] wherein said DNA-DNA or DNA-RNA hybridization

occurs under 2X PIPES buffer, 50% deionized formamide, 0.5% (w/v) SDS, 500μg/ml denatured sonicated salmon sperm DNA at 42°C overnight; and said DNA fragment or its complement remains hybridized after washing in 2X SSC, 1% (w/v) SDS;

(3) said DNA fragment encodes the part of said protein in which an amino acid corresponding to Val13 of SEQ ID NO:1 [or SEQ ID NO:2 or SEQ ID NO:3] is substituted by another amino acid; and

(4) said DNA fragment has an ability to confer resistance to protoporphyrinogen oxidase-inhibiting herbicides in plant or algal cells when expressed therein.

21. (Twice Amended) The isolated DNA fragment according to any of claims [15 to 20,] <u>15, 16, 18 and 20,</u> wherein said another amino acid is methionine.